

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

HARTLEY *et al.*

Appl. No. *To be assigned*

(Continuation of U.S. Appl. No. 09/177,387)

Filed: HERewith

For: **Recombinational Cloning Using  
Nucleic Acids Having  
Recombination Sites**

Confirmation No.: *To be assigned*

Art Unit: *To be assigned*

Examiner: *To be assigned*

Atty. Docket: 0942.285000D/BJD/MTT

**Preliminary Amendment**

Commissioner for Patents  
Washington, D.C. 20231

Sir:

In advance of prosecution of the captioned application, Applicants submit the following Preliminary Amendments and Remarks. This Preliminary Amendment is provided in the following format:

- (A) A clean version of each replacement paragraph/section/claim along with clear instructions for entry;
- (B) Starting on a separate page, appropriate remarks and arguments. 37 C.F.R. § 1.121 and MPEP 714; and
- (C) Starting on a separate page, a marked-up version entitled: "Version with markings to show changes made."

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and

any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

***Amendments***

Please amend the application as follows:

***In the Specification:***

In the specification at page 1, please delete the first full paragraph (appearing at lines 6-11), and substitute therefor the following paragraph:

The present application is a CONTINUATION of U.S. Application No. 09/177,387 filed on October 23, 1998, which claims the benefit of the filing date of U.S. Provisional Application No. 60/065,930, filed October 24, 1997, the disclosures of which are incorporated by reference herein in their entireties. The present application is also related to U.S. Application No. 08/663,002, filed June 7, 1996, and to U.S. Application No. 08/486,139, filed June 7, 1995, the disclosures of which applications are incorporated by reference herein in their entireties.

***In the Claims:***

Please amend the claims as follows:

- (a) Please cancel claims 2-51, without prejudice to or disclaimer of the subject matter contained therein. Applicants reserve the right to prosecute the subject matter of these claims in one or more continuing applications.
- (b) Please enter the following new claims 52-67:

52. (New) A method for cloning or subcloning an amplified nucleic acid molecule comprising:

- (a) amplifying a nucleic acid template with a first primer comprising at least a first recombination site and a second primer comprising at least a second recombination site, wherein said first and second recombination sites do not recombine with each other, under conditions favoring the production of a product nucleic acid molecule complementary to all or a portion of said template and comprising said first and second recombination sites; and
- (b) combining said product nucleic acid molecule with at least one vector comprising at least a third and a fourth recombination sites that do not recombine with each other, under conditions such that recombination occurs between said first and third and said second and fourth recombination sites, thereby producing a product vector.

53. (New) The method of claim 52, wherein said amplification is accomplished by PCR.

54. (New) The method of claim 52, further comprising inserting said product vector into a host cell.

55. (New) The method of claim 52, wherein said vector is an expression vector.

56. (New) The method of claim 52, wherein said vector comprises at least one additional nucleic acid sequence selected from the group consisting of a selectable marker, a cloning site, a restriction site, a promoter, an operon, an origin of replication, and a gene or partial gene.

57. (New) The method of claim 52, wherein said vector comprises at least one origin of replication.

58. (New) The method of claim 52, wherein said vector comprises at least one promoter.

59. (New) The method of claim 52, wherein said vector comprises at least one selectable marker.

60. (New) The method of claim 52, wherein said nucleic acid molecule and said vector are combined *in vitro*.

61. (New) The method of claim 52, wherein said product nucleic acid molecule is linear.

62. (New) The method of claim 52, wherein said first, second, third or fourth recombination sites are *lox* sites or mutants thereof.

63. (New) The method of claim 62, wherein said *lox* sites are selected from the group consisting of *loxP* sites and *loxP511* sites.

64. (New) The method of claim 52, wherein said first, second, third or fourth recombination sites are *att* sites or mutants thereof.

65. (New) The method of claim 64, wherein said *att* sites are selected from the group consisting of *attB* sites, *attP* sites, *attL* sites and *attR* sites.

66. (New) The method of claim 52, wherein said first, second, third or fourth recombination sites are selected from the group consisting of a *lox* site, an *att* site, an FRT site, and mutants thereof.

67. (New) The method of claim 52, wherein said product nucleic acid molecule and said vector are combined in the presence of at least one recombination protein.

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**Remarks**

By the foregoing amendments, claims 2-51 have been cancelled without prejudice or disclaimer, and new claims 52-67 are sought to be entered. Support for the foregoing amendments to the claims may be found throughout the specification. Specifically, support for the new claims may be found, *inter alia*, at pages 6-7, 11-13, 20-24, 27-38, 46-47, and throughout the Examples, particularly at pages 51-53 and 71-76. Accordingly, the present amendments do not add new matter, and their entry is respectfully requested. Upon entry of the foregoing amendments, claims 1 and 52-67 are pending in the application, with claims 1 and 52 being the independent claims.

It is believed that the present application is in condition for immediate examination. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



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P104-59.wpd

**Version with markings to show changes made**

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***In the Claims:***

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- (b) New claims 52-67 are sought to be added.